# Effect of Preparate Coloring Delay Achid Resistant Bacteria With Ziehl Neelsen Method On The Result of Microscopic Examination

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#### Abstract.

The microscopic examination of smear from sputum specimens plays an important role in the initial diagnosis and monitoring of pulmonary TB treatment. The Ziehl Nelseen method is the method for examining acid-resistant bacteria smear recommended by WHO. This method has high specificity for detecting acid-resistant bacteria in sputum. This study aims to determine the effect of postponement of sputum smear staining on the quality of acid-resistant bacteria germs on the results of microscopic examination using the Ziehl Nelseen staining method. The length of time to delay staining used was 3 days, 2 days and the control was direct preparations staining. The sample used was positive smear sputum. The results showed that from 10 samples of sputum, the results obtained were directly stained with results of 1+ there were 7 samples, and 2+ there were 3 samples, while the samples with the preparations were delayed for 2 days and 3 days at room temperature, respectively +1 as many as 7 samples, and 2+ as many as 3 samples. However, there was no difference in the reading of the staining results between the direct stained samples with the stained preparations with a delay of 2 days and 3 days at room temperature. The results of this study still have many shortcomings and to improve them it is recommended to carry out further research with a larger scale and sample size.

**Keywords:** Acid-resistant bacteria, Preparation, Tubercolosis

#### 1. INTRODUCTION

Pulmonary Tuberculosis (Pulmonary TB) is an infectious disease that is still a problem in society, both in terms of mortality (mortality), disease incidence (morbidity), as well as diagnosis and treatment [1]. Pulmonary TB is listed as one of the world's health problems included in the Millennium Development Goals (MDGs), ranking second as a killer disease after Human Immunodeficiency Virus in cases of infectious diseases [2, 3]. And until now, Indonesia as a developing country is still one of the countries with the highest pulmonary TB cases in the world [4].

Pulmonary TB is an infectious disease and attacks the respiratory system, especially the parts of the lungs caused by Mycobacterium Tuberculosis [5]. Mycobacterium Tuberculosis is a type of gram-positive bacteria that lives in the environment and then enters the inhalation of the respiratory system [6]. These bacteria

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can be transmitted from one individual to another through droplet droplets carried by the air such as coughing, sputum or saliva [7]. Sputum droplets are very small in size, can pass through the mucociliary defense system of the bronchi, and keep walking until they reach the alveoli and settle there. Infection begins when bacteria multiply by dividing themselves in the lungs, resulting in inflammation in the lungs. The time between the occurrence of infection to the formation of the primary complex is about 4-6 weeks [8].

The main target of controlling pulmonary tuberculosis (TB) is to find infectious TB patients (smear positive) and cure the disease. By prioritizing the detection of TB patients with positive smear, the laboratory is the main key in diagnosing TB patients. This is emphasized in the second component of the DOTS strategy, namely diagnosis using microscopic examination. The microscopic examination of smear from sputum specimens plays an important role in the initial diagnosis and monitoring of pulmonary TB treatment. The Ziehl Nelseen (ZN) method of AFB examination is the method recommended by WHO. This examination method is still the first choice for early detection of TB. The ZN technique is an easy, inexpensive, and high specificity technique for detecting acid-resistant bacteria (BTA) in sputum. A good series of activities is needed to get accurate results, starting from how to collect sputum, selecting the sputum material to be examined, making sputum smears, staining techniques and tools or materials / reagents that must be considered for quality [9].

In line with the level of public awareness to check themselves in health service places, including the Puskesmas, the number of sputum specimens that will be examined by the puskesmas laboratory will certainly increase and require the alertness of laboratory staff. The condition of the sputum in the microscopic examination of AFB is important. Good sputum contains some particles or is slightly thick and slimy, sometimes even festering or yellowish green [10]. To ensure good quality sputum specimens, they should be sent to the laboratory as soon as possible after collection. However, if the sputum that has been taken from the patient is piled up and waiting for a laboratory examination, it can certainly reduce the quality of the sputum and affect the results of the microscopic examination readings [11].

To prevent this, a Health Analyst tries to maintain the quality of sputum by immediately making sputum slides for examination, but sometimes due to limited time and human resources, the sputum slides that have been fixed have to be stored and postponed for hours or even days. before staining. Therefore, researchers are interested in studying and conducting research on the effect of postponement of the Ziehl Neelsen method of AFB staining on the results of microscopic examination, to determine the effect of postponement of positive smear preparations on the quality of BTA in sputum.

# II. METHODS

This research is a laboratory experimental research. Performed Ziehl Neelsen's stain on positive smear sputum with a long delay period for staining different preparations.

The sample used in this study was 10 (ten) positive smear sputum from pulmonary tuberculosis patients, where the sputum sample would be divided into 3 treatment groups, namely a 2-day delay in staining preparations at room temperature, a 3-day delay in staining preparations at room temperature, and control. (without delay treatment of preparations staining). The research was conducted at the Microbiology Laboratory of the Mamajang Health Center, Makassar City. Sputum samples were taken from patients with a diagnosis of pulmonary tuberculosis who came for examination at the Puskesmas, and met the predetermined inclusion criteria. Inclusion criteria: dilute sample, cough for more than three weeks. Exclusion criteria: dilute sample, saliva sample. The sputum samples used in this study were limited to morning sputum obtained from patients who came to the Puskesmas in person. Each sample was examined using the Ziehl Neelsen (ZN) staining technique.

In this study, the preparation of a sputum smear was made with a loop or loop, in the following order: ose was heated over a Bunsen flame until it was red and allowed to cool, for sterilization. Sputum is spread evenly in an oval shape on the surface of the slide with a size of about 2 x 3 cm. The dry preparation is taken with tweezers on the side labeled with the sputum preparation facing up. Then the preparation is fixed by passing it over a flame 2-3 times.

Each sputum sample was made in 3 types of preparations (sputum smear preparation) on 3 different glass objects for 3 different treatment groups. Treatment group 1: sputum smear that has been dry on the glass object then fixed and left at room temperature for 2 days (2 x 24 hours) before ZN staining, Treatment group 2: sputum smear preparation that has been dry on the glass object then fixed and left at room temperature for 3 days (2 x 24 hours) before ZN staining, and treatment group 3 (as a control): sputum smear that was dry then fixed and immediately carried out ZN staining.

The ZN staining technique was carried out by adding a 0.3% carbol fuchsin solution to the entire surface of the preparation (sputum smear). After that, it is heated over a flame until it comes out smoke (but not boiling or dry) for 5 minutes. The preparation is allowed to dry in the air for 5-7 minutes. Excess paint is removed and washed under running water. After that, the preparation is given a 3% hydrochloric acid-ethanol solution and left for 2-4 minutes then washed with running water for 1-3 minutes. After that, 0.1% methylene blue solution was poured to cover the entire surface and waited for 1 minute then the solution was discarded and washed with running water, then dried and the results were read.

The reading of the staining was carried out with a 1000x magnification microscope by dropping emersion oil on the preparation. BTA bacteria will be brick pink and non-BTA bacteria will appear blue. Reading of the results of the number of AFB based on the IUATLD scale (International Union Against Tuberculosis and Lung Diseases, Table 1).

**Table 1.** Scale of IUATLD (International Union Against Tuberculosis and Lung Diseases)

Readings under the microscope	Reporting of Results		
BTA was not found in 100 laps of vision	Neg.		
1-9 BTA in 100 fields of view.	Write down the number of BTA found.		
10-99 BTA in 100 fields of view.	+1 (positive 1)		
1-10 BTA in 1 field of view.	+2 (positive 2)		
> 10 BTA in 1 field of view	+3 (positive 2)		

#### III. RESULT AND DISCUSSION

Examination of sputum specimens (sputum) microscopically is identical to the examination of sputum culture. Microscopic examination of sputum is still considered efficient, easy, cheap, specific and sensitive. Supporting the success of the sputum microscopic test is the quality of the sputum so that false negative smear results are not obtained. From the research that has been carried out regarding the effect of delaying the staining of acid-resistant bacterial preparations of the Ziehl Neelsen method on the results of microscopic examination, the results are as shown in table 2.

**Table 2.** The results of staining using several time delays in the staining of smear preparations

NO.	The Quality of ZN Staining Results		
	Preparation Delay 3 Days	Preparation Delay 3 Days	Control
1	Positive 1	Positive 1	Positive 1
2	Positive 1	Positive 1	Positive 1
3	Positive 1	Positive 1	Positive 1
4	Positive 2	Positive 2	Positive 2
5	Positive 1	Positive 1	Positive 1
6	Positive 1	Positive 1	Positive 1
7	Positive 2	Positive 2	Positive 2
8	Positive 1	Positive 1	Positive 1
9	Positive 2	Positive 2	Positive 2
10	Positive 1	Positive 1	Positive 1

From table 2 it can be seen that the 10 sputum samples used as subjects in this study gave positive BTA staining results with good and clear staining results (light brick red). In the sputum sample that was directly stained (as a control), 7 samples were obtained positive results 1, and 3 samples positive results, while the samples with the coloring preparations were delayed 2 days and 3 days at room temperature, respectively obtained a positive result 1 of 7 samples, and positive 2 as many as 3 samples. This shows that there is no difference in the reading of the staining results between the direct staining samples with the staining of the preparations with a delay of 2 days and 3 days at room temperature. There are no results with increasing numbers (positive) and no results (0). This means that the examination of sputum preparations delayed 2 days and 3 days at room temperature did not result in false negative and false positive results.

## IV. CONCLUSION

Based on the research results obtained by doing Ziehl Neelsen staining on sputum smear preparations with different delay time durations, it can be concluded that there was no difference between the results of BTA staining on the sputum smear preparations that were delayed for 2 days and 3 days at temperature. space with the stained result of the sputum smear preparation which is directly colored. This means that the examination of the sputum preparations was delayed by 2 days and 3 days at room temperature did not result in false negative and false positive results. The results of this study still have many shortcomings and to improve them it is recommended to carry out further research with a larger scale and sample size.

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